ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Twenty-seventh Quarterly Report of Progress

Research Project R-36-015-001

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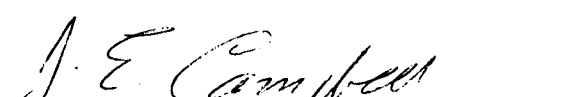

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Introduction

In the 25th Quarterly Report of Progress, we identified a set of conditions in which 90°C was a more lethal temperature than 125°C for the destruction of Bacillus subtilis var. niger. In the 26th Quarterly Report of Progress, we demonstrated that at 90°C the lethality of the system increased as a function of relative humidity, with maximum effectiveness at 100% R.H. In this report, we present a systematic study of the influence of head-space moisture and temperature on the destruction of B. subtilis var. niger as an extension of previous observations.

I. EXPERIMENTAL

All experiments reported here were carried out in our conventional system. The spores were suspended in 95% ethyl alcohol, diluted in sterile double-distilled water, and dispensed with a repeating dispenser in 0.1-ml amounts in stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 mm x 300 mm tin cans. Thirty cups were on each shelf and four shelves were used in each can for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 minutes at 45 to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every

10 minutes for the first 70 minutes, and this was followed by five consecutive purges of nitrogen with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood. Appropriate amounts of water were placed in each can. The cans were sealed and removed from the equilibration hood. Heat treatments were applied as discussed below.

Spore survivors were assayed by sonifying the cups in peptone water, and plating and counting on TGE agar. Prior to heat treatment, the seams on each can were soldered and wiped to preclude leakage of water vapor during heating cycle.

II. RESULTS AND DISCUSSION

Data are presented in Figure 1 showing the influence of temperature on the destruction of B. subtilis var. niger at 100% relative humidity. As would be expected, the time required to destroy the organism was inversely proportional to the temperature. However, the shape of the destruction curve was of particular interest in that the difference in time between the highest and lowest temperature is largely the difference in the induction period prior to destruction. The rate of destruction of the organism once begun appears not to be greatly affected by temperature.

These findings are in marked contrast to those obtained under dry conditions (relative humidity of about 0.02% at 125°C) in which the rate of destruction decreases as a function of time (see Figure 3, 20th Quarterly Report of Progress).

Although we have not undertaken any systematic studies on mechanism of kill, it would appear, from the differences between the inactivation curves under high and low relative humidity, that we are dealing with two distinctly different mechanisms of thermal inactivation.

The next series of experiments investigating the influence of head-space moisture are summarized in Figure 2. The heating time selected for each temperature was the time required to kill approximately 6 logs of B. subtilis var. niger at 100% relative humidity. It is seen from these data that as the temperature was increased, the amount of water that must be added to the can to obtain equivalent kill was increased. If, however, the log of survivors are plotted against the relative humidity (Figure 3) at each temperature, they become a family of close-parallel curves. The data clearly show that the resistance of B. subtilis var. niger decreases directly as a function of relative humidity from 50 to 100%. A demonstration of this phenomonon is shown in Figure 4, in which the destruction of the test organism is plotted against time under conditions of 100% and 80% R.H.

III. PROPOSED RESEARCH FOR THE NEXT QUARTER

a. Undertake studies on the nature of moisture in spores.

b. Explore mechanism of thermal inactivation under conditions of high and low findings.

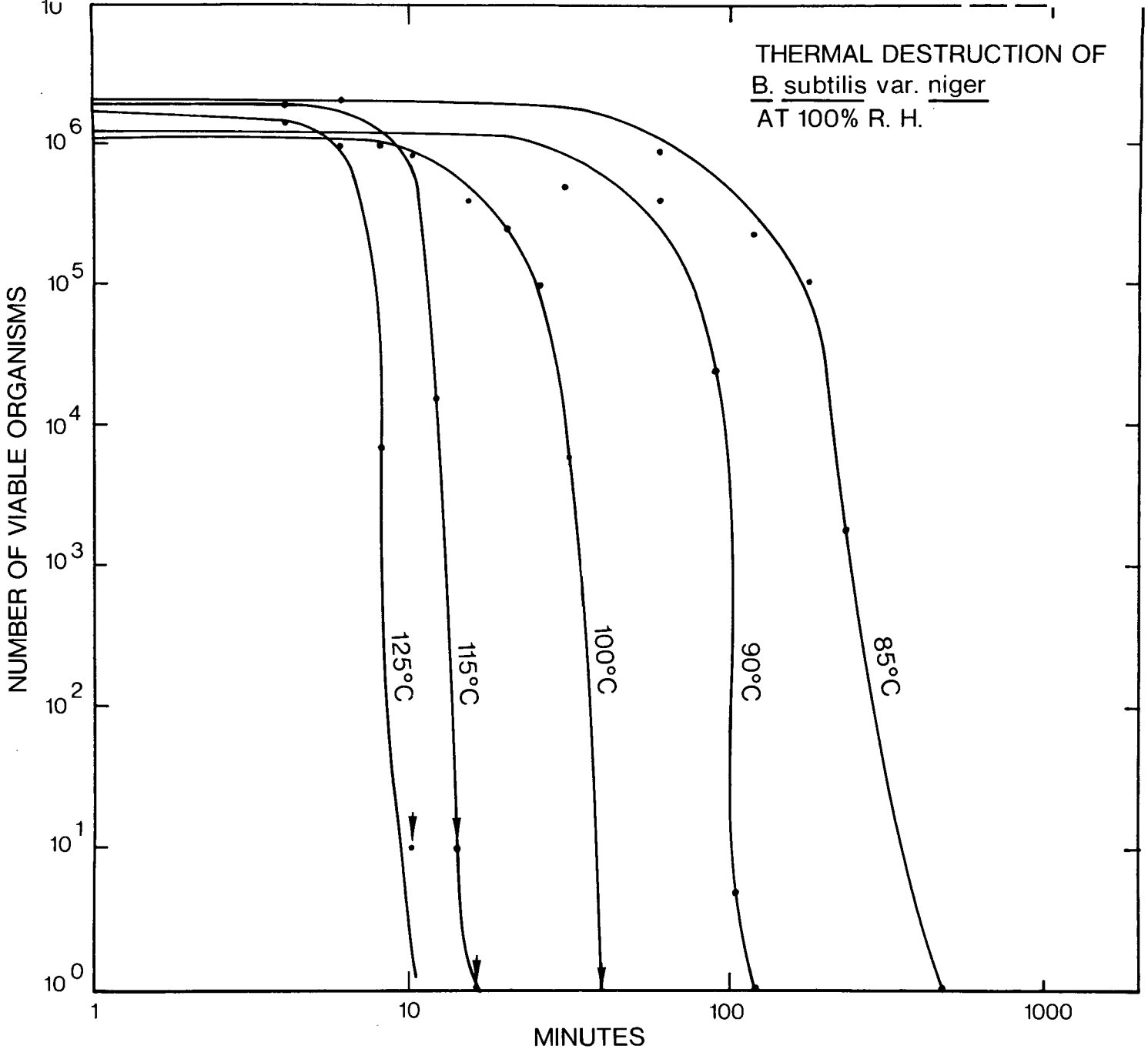
THERMAL DESTRUCTION OF
B. subtilis var. niger
AT 100% R. H.
NUMBER OF VIABLE ORGANISMS
MINUTES
125°C
115°C
100°C
90°C
85°C
10
10^6
10^5
10^4
10^3
10^2
10^1
10^0
1
10
100
1000

FIGURE 1.

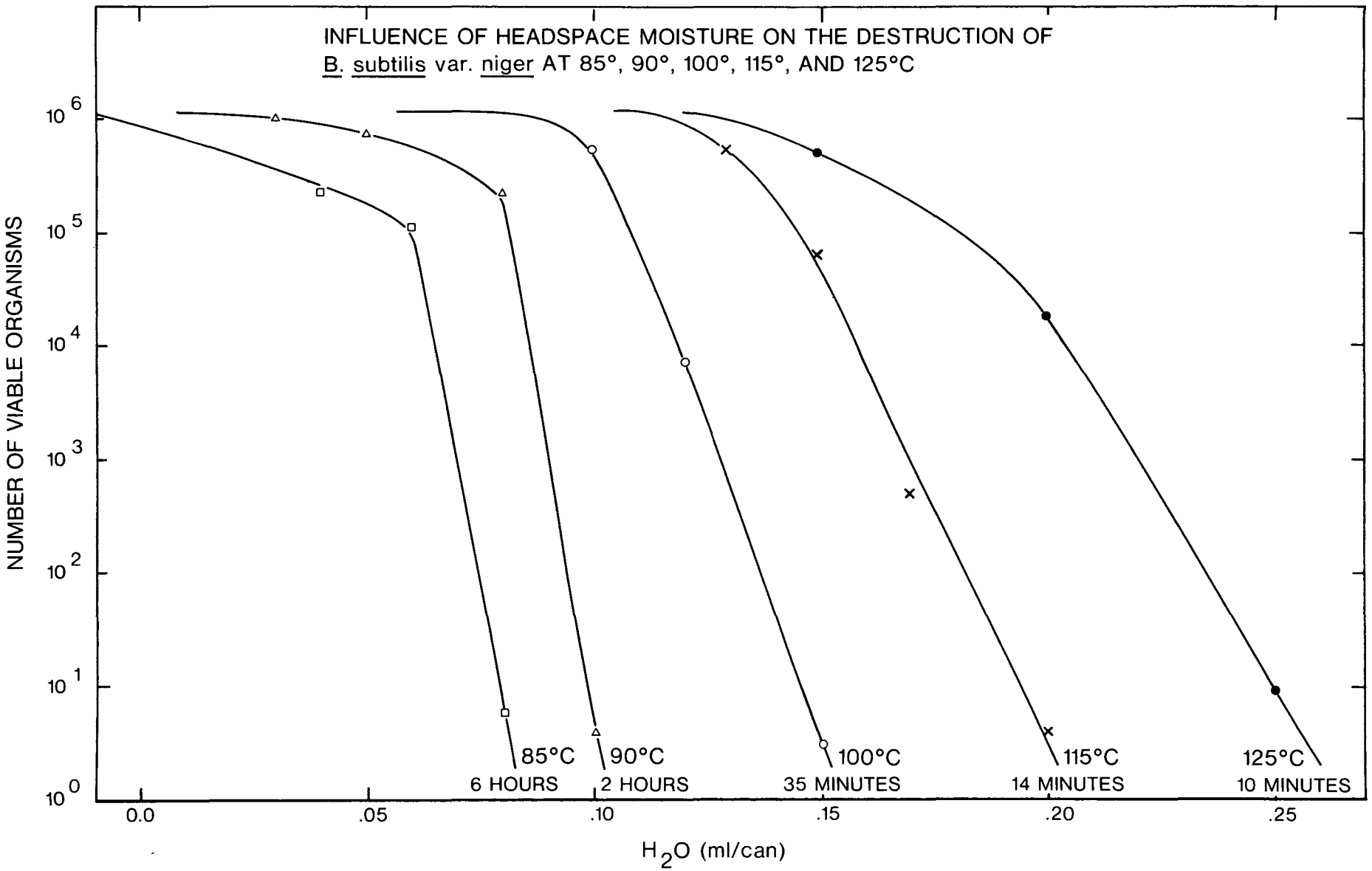
INFLUENCE OF HEADSPACE MOISTURE ON THE DESTRUCTION OF
B. subtilis var. niger AT 85°, 90°, 100°, 115°, AND 125°C
NUMBER OF VIABLE ORGANISMS
10 6
10 5
10 4
10 3
10 2
10 1
10 0
0.0
.05
.10
.15
.20
.25
H2O (ml/can)
85°C
6 HOURS
90°C
2 HOURS
100°C
35 MINUTES
115°C
14 MINUTES
125°C
10 MINUTES

FIGURE 2.

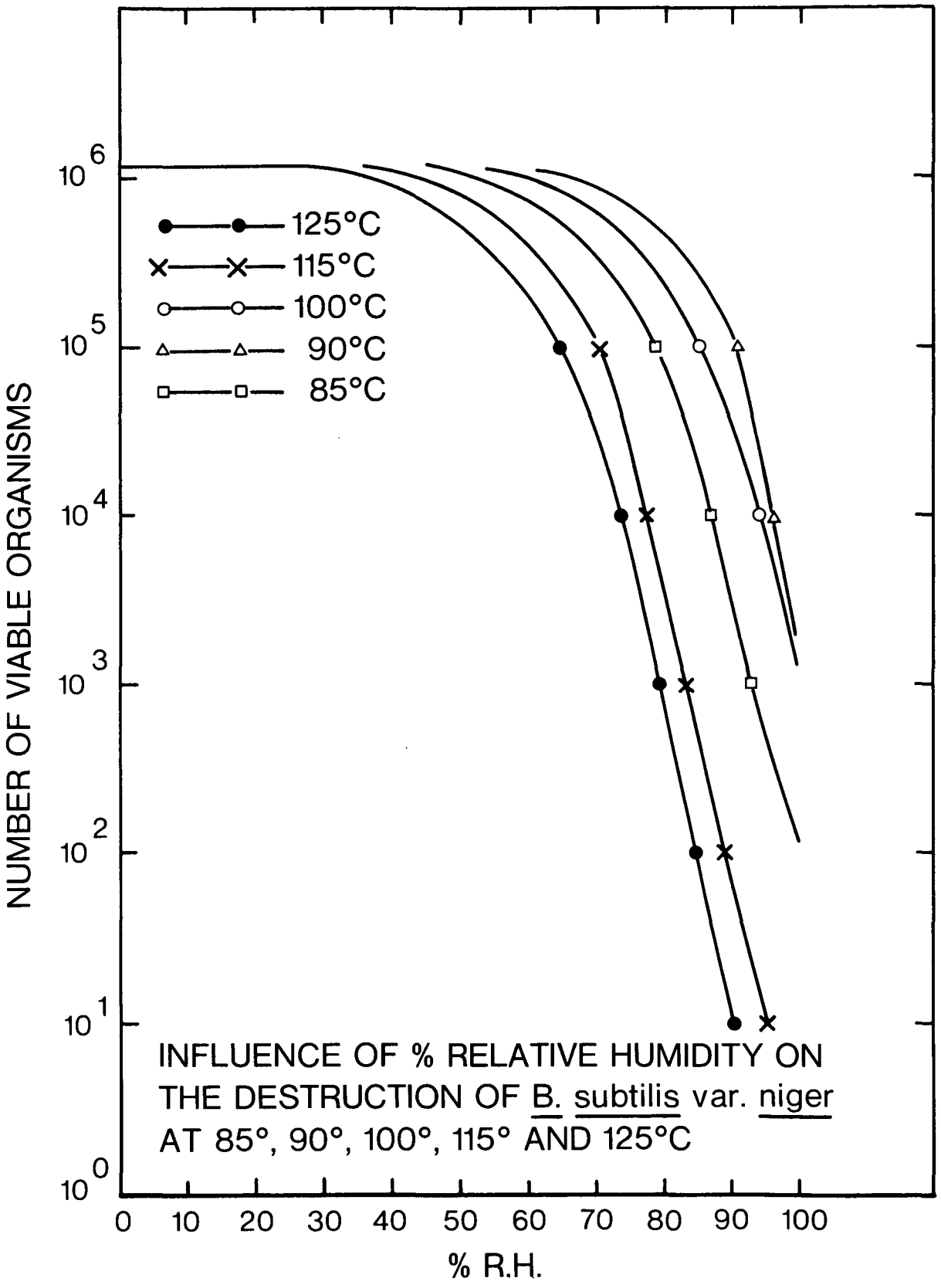
NUMBER OF VIABLE ORGANISMS
10^6
10^5
10^4
10^3
10^2
10^1
10^0
125°C
115°C
100°C
90°C
85°C
INFLUENCE OF % RELATIVE HUMIDITY ON
THE DESTRUCTION OF B. subtilis var. niger
AT 85°, 90°, 100°, 115° AND 125°C
0 10 20 30 40 50 60 70 80 90 100
% R.H.

FIGURE 3.

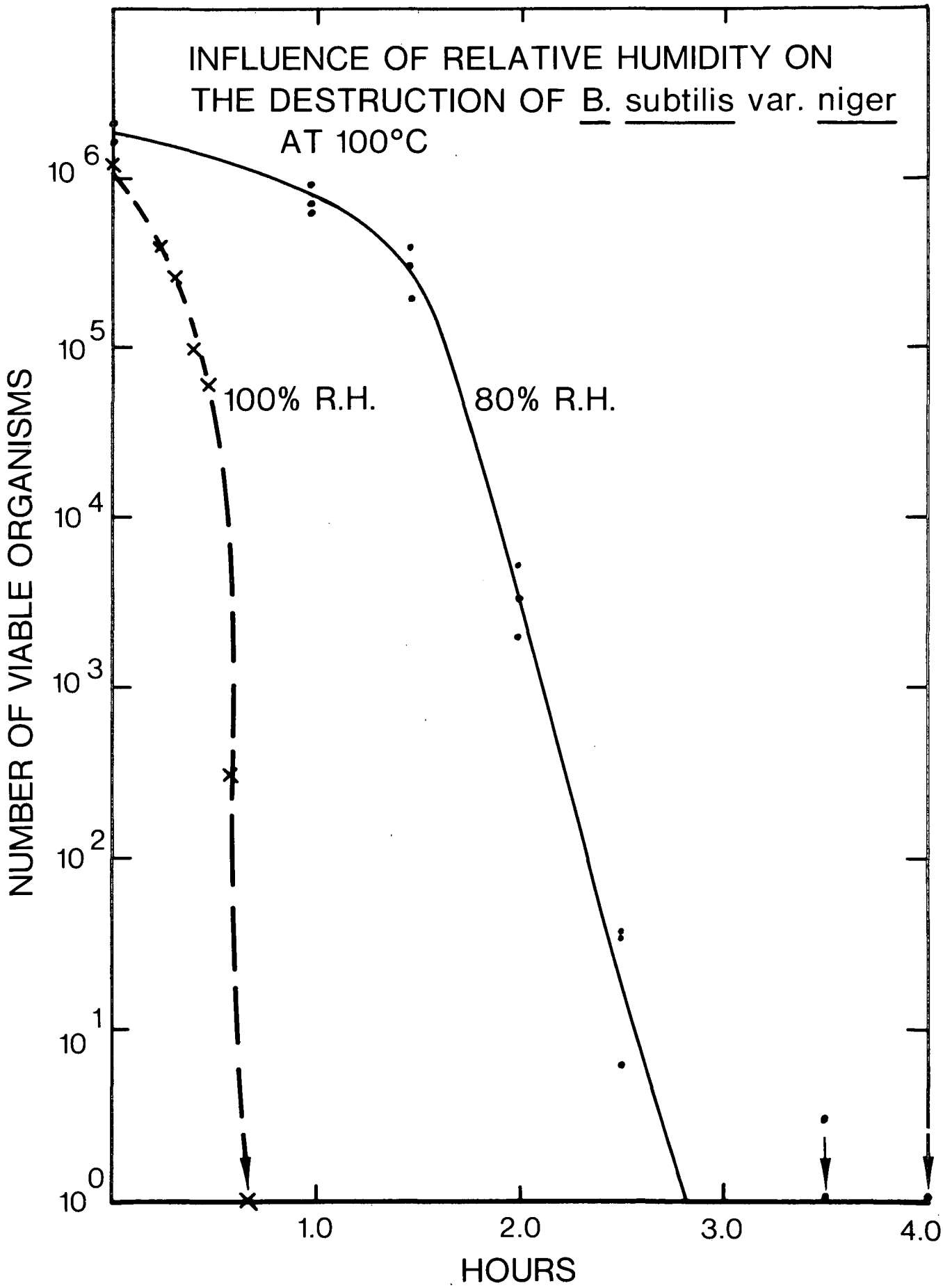
INFLUENCE OF RELATIVE HUMIDITY ON
THE DESTRUCTION OF B. subtilis var. niger
AT 100°C
NUMBER OF VIABLE ORGANISMS
10 6
10 5
10 4
10 3
10 2
10 1
10 0
100% R.H.
80% R.H.
1.0
2.0
3.0
4.0
HOURS

FIGURE 4.